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IONTOPHORETIC STUDIES OF NEURONES IN THE MAMMALIAN CEREBRAL CORTEX

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The interpretation of studies of the actions of various chemical agents on the cerebral cortex is peculiarly fraught with difficulties. Substances injected into the blood circulation may never reach cortical neurones on account of the blood–brain barrier, and they are quite likely to produce indirect effects by altering the blood supply to the brain, or by exciting peripheral structures with central connexions. Direct application to the surface of the cortex may not give a more definite answer, because excitability is abolished by inhibition or an excess of excitation and, in general, changes in electrical records from the surface can be quite ambiguous (compare the analyses of the action of γ-aminobutyric acid (GABA) by Purpura, Girado, Smith, Callan & Grundfest, 1959, on the one hand, and by Jasper, 1960, and Goldring & O'Leary, 1960, on the other hand). The cortex is not a homogeneous structure. Different neurones may not respond to drugs in the same way, but this need not be revealed by recording the activity of single units, since the numerous excitatory and inhibitory interconnexions between neurones may cause the reaction of the single unit to be altered radically by the massive discharge of other cells.

For all these reasons the iontophoretic method of applying substances to single units is the method of choice whenever the substance is ionized in solution. The amounts released are minute and therefore the concentration in the tissue negligible, except in the immediate vicinity of the neurones under observation. Many neurones can thus be studied in succession, in the same animal, little interference with the function of the brain being caused.

Iontophoresis has been used extensively in experiments on spinal neurones (e.g. Curtis & Eccles, 1958; Curtis, Phillis & Watkins, 1959, 1960) and also in two studies of neurones in the brain stem (Curtis & Koizumi, 1961, and Curtis & Davis, 1962).

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In the present paper we shall describe the excitation and inhibition of single cortical neurones by various agents applied iontophoretically, considering in particular the remarkable actions of dicarboxylic and ω-amino monocarboxylic acids, originally discovered by Hayashi (1954, 1956), as well as other interesting phenomena, such as the excitation of certain neurones by acetylcholine (ACh) and some effects produced by indole and catechol amines.

Brief reports of our results have already appeared (Krnjević & Phillis, 1961*a*, *b*).

METHODS

Experiments were performed on cats, rabbits and rhesus monkeys. In most cases the animals were anaesthetized with Dial compound, containing diallyl-barbituric acid (0·1 g/ml.) and urethane (0·4 g/ml.) (Ciba Ltd.), injected intraperitoneally (0·7 ml./kg). This was sometimes supplemented with intravenous pentothal sodium or sodium pentobarbitone (Nembutal; Abbott Laboratories). In a few experiments cats were anaesthetized with ethyl chloride and ether, and then either kept on ether, or given intravenous chloralose (80 mg/kg).

To eliminate a possible interference by anaesthetics, in four experiments we examined neurones in the unanaesthetized 'cerveau isolé' of the cat. This was prepared under ether, either as in the original description by Bremer (1937), i.e. by a section of the brain stem along the plane of the bony tentorium, or by a more inclined section (at 45°) beginning just behind the base of the tentorium. Neither approach was successful in preventing the frequent occurrence of cerebral oedema when the cortex was subsequently exposed. As a rule we made a wide exposure, to allow recording from different regions, including the symmetrical areas of both hemispheres. To prevent drying and to reduce cooling, the cortex was covered with one layer of transparent polythene and a second layer of black polythene (polythene mulch, Fablon). The area left free for the insertion of the micropipette was irrigated by a continuous drip of warm physiological fluid. The whole animal was kept at $37 \pm 1°$ C by an automatically controlled heating pad under its abdomen (Krnjević & Mitchell, 1961).

The multibarrelled pipettes consisted of five hard glass tubes (diameter 6·5 mm, bore 4·5 mm) fused together for part of their lengths; the array of five tubes was heated and drawn out to a fine tip by a device similar to that described by Winsbury (1956). The micropipettes were filled by boiling in glass-distilled water. Before replacing the water with concentrated (nearly saturated) solutions of various substances, the shaft was broken back to give a tip diameter of 6–12 μ. This was done to ensure that barrels do not have an excessively high electrical resistance: as a rule, after filling with concentrated solutions of the various substances, the barrel resistances were in the order of 20–100 MΩ. In all the micropipettes the central barrel (which was always wider than the others, with a resistance only about one tenth as high for a given internal solution) was filled with 2·7 M-NaCl; it was connected by a short silver wire to the grid of the cathode follower at the input of the amplifying system. One barrel was usually filled with Na L-glutamate for the activation of quiescent neurones. Whenever we studied cholinoceptive cells, another barrel contained acetylcholine chloride (AChCl; Roche Products) at a concentration of about 3 M. Thus either two or three barrels were available for the various substances to be tested. They were made in approximately saturated solutions; for amino acids, strong acid or alkali was usually added to bring the pH well away from the iso-electric point, in most cases as in the experiments of Curtis & Watkins (1960).

Each of the barrels used for iontophoresis was connected by a flexible lead and a 60 MΩ resistor to a separate polarizing battery, the return circuit being completed through earth and an Ag–AgCl earthing electrode wrapped in moist gauze and fixed to muscles on the back

of the neck. The polarizing currents could be varied and reversed, and their intensity was monitored continuously on a series galvanometer, with an accuracy of ± 1 nA. Provision was also made for the constant application of a suitable braking current to neutralize the spontaneous outflux of substances from the tip of the pipettes (cf. del Castillo & Katz, 1955). The position of the micropipette was controlled by micromanipulator which allowed vertical movements in steps of 1 μ. To reduce pulsations a transparent Perspex pressor was applied to the surface of the brain with no impairment of blood flow through underlying cortical vessels (Phillips, 1956). Penetration by the micropipette was made easier by disruption of the pial layer, performed with fine forceps under a microscope.

The electrical activity of single neurones was amplified in the conventional manner. Spikes on a slow time base were easily photographed thanks to the excellent beam intensifier described by Pokrovsky (1960).

RESULTS

We have made observations on well over 4000 cortical units, in 56 cats, 4 rabbits and 4 monkeys. Most of them were recorded in the cerebral cortex, but in 5 experiments units were also examined in the cerebellar cortex of cats and rabbits.

Current effects

Iontophoresis depends on a flow of current into or out of the tip of the pipette. Since the tip must be very near a cell to record its activity, how much is the activity of the cell affected by the flow of current? This question is best answered by observing the effects of currents flowing from a multi-barrelled pipette containing solutions of NaCl in all its barrels. Under these conditions we found that most cells could be excited to some extent by a large inward current (i.e. one flowing from the tissue into the relatively negative tip of the micropipette) and conversely, they could be depressed by a large outward current (i.e. with tip positive). For a definite action, currents of at least 100 nA were usually needed.

The effect was seen best when the cell was discharging spontaneously, and therefore already in an excitable state. For instance, the unit in Fig. 1*B* was initially discharging spontaneously at a relatively high rate, but its activity could be blocked by an outward current of 100 nA. A few minutes later the same unit became much less active, and at this time an inward current of 120 nA had a sharp excitant action (Fig. 1*A*). Such powerful current effects were somewhat unusual, but these records demonstrate clearly several characteristic features: the instantaneous onset and termination (cf. the actions of various substances in Figs. 3, 5, 6, 9, 10, 12, 17, etc.), the tendency to adaptation, causing the effect to remain maximal for only 2–3 sec, and the rebound of excitability, lasting up to 15 sec after turning off the current.

On a few occasions we have seen current effects in the reversed direction: i.e. with excitation by an outward current and depression by an inward

current (Fig. 1*C* and *D*). These were associated with evidence that the tip of the pipette was extremely close to the cell membrane: spikes were positive and small currents unusually potent; there was a tendency to injury discharge, and electrical artifacts arising from movements. On account of the relatively large tips of our pipettes, we could not record very long under these conditions; the unit soon either reverted to the normal behaviour or it discharged paroxysmally and was lost.

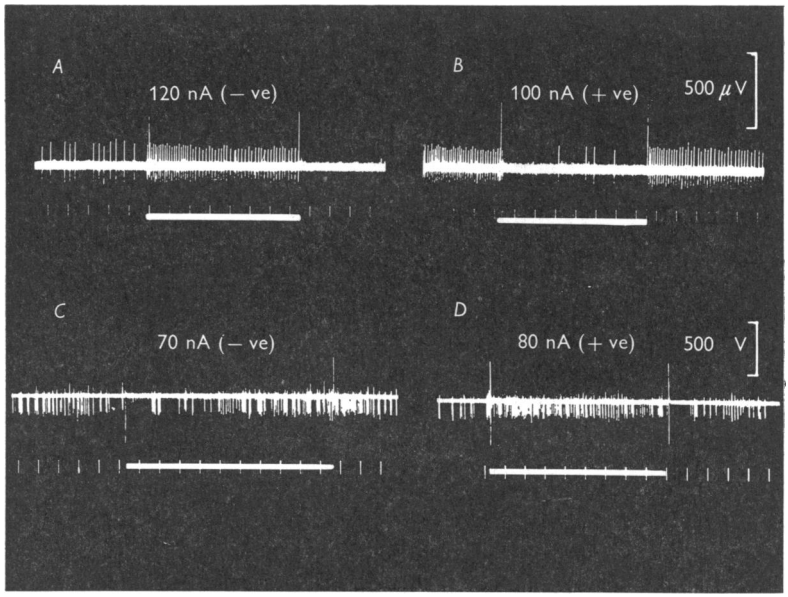

Fig. 1. Effects of current flow on cortical neuronal activity in cats. *A*, *B*: unit in cerveau isolé excited by an inward current (tip negative) and depressed by an outward current; pipette contained NaCl in all barrels. *C*, *D*: unit in animal under Dial giving positive spikes and showing exceptional effects of current flow, opposite to those in *A*, *B*. Note instantaneous changes in activity and tendency to accommodation and rebound.

Although current effects are probably never entirely negligible, they do not interfere to any serious extent with iontophoretic studies. The actions of powerful excitatory or inhibitory substances are hardly affected at all; at most there may be some tendency towards facilitation or depression, depending upon whether the substance is released as an anion or a cation, and the time course of the action may be slightly modified. On the other hand, the excitation or depression produced by less active substances might not be so readily distinguished from current effects. But with multi-barrelled pipettes, indifferent barrels are usually available, through which similar or greater currents can be passed as a control. This

procedure was done as a routine whenever there was the slightest doubt about the action of any substance (as shown in Figs. 2, 7, 10 and 17). Cerebellar cortical neurones seemed rather more sensitive to current effects than neurones in the cerebral cortex.

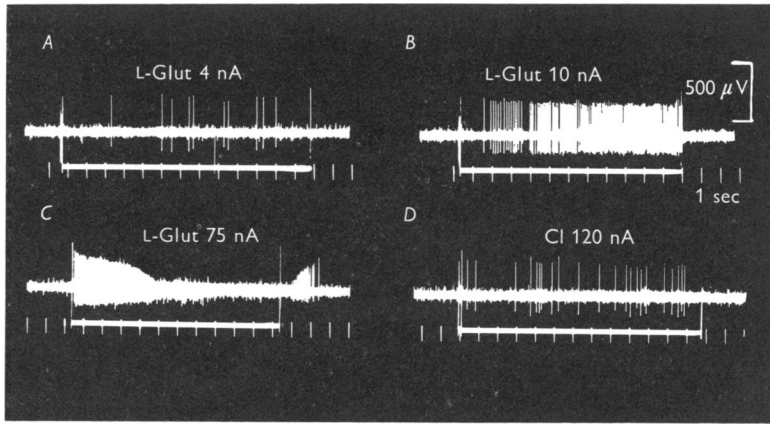

Fig. 2. Excitation by L-glutamate of unit in cerebellar cortex of cat under Dial. *A–C*: white lines indicate iontophoretic application by inward currents (tip negative). *D*: control for current effects; large inward current was passed through adjacent barrel containing AChCl.

Excitation by inward currents appeared to be somewhat greater if one of the other barrels contained L-glutamate. This was probably owing to some accumulation of glutamate by spontaneous outflux in the region just outside of the tip, where the four barrels have a common external resistance. Inward current would tend to carry some of this glutamate towards the cell. If the current is passed through one barrel, there is no appreciable current flow in the same direction through adjacent barrels; thus ACh+ is not released from one barrel by a very large current through an adjacent barrel (Krnjević, Mitchell & Szerb, 1963). A small current does pass in the opposite direction in the other barrels because they offer a parallel shunt to earth, via the polarizing circuits. However, this path has such a high electrical resistance (at least 80 MΩ for each barrel) when compared with the common external resistance (1–100 kΩ) as to be of negligible significance. Non-specific interference by currents is probably greatest when an outward current carrying one substance is superimposed on an inward current releasing L-glutamate. This is a convenient method of testing depressant substances; but it is clear that part of the outward current will tend to flow into the glutamate-containing barrel, reducing the outward transport of glutamate.

Excitation by L-*glutamate and certain related amino acids*

Identification of excitable units. As far as we can tell, all cortical neurones are excited by L-glutamate. Since we cannot observe the tip of the micropipette, our identification of the structures excited must rest on certain indirect considerations. Excitable units were found in large numbers at all depths of the cortex, but only occasionally in white matter, and most of them generated stable and substantial extracellular spikes recorded over relatively large distances (up to 100 μ). These facts suggest that L-glutamate excites the cell bodies (and perhaps large dendrites) of cortical neurones. We cannot tell at present whether some or perhaps all glial cells also respond to L-glutamate, but it is difficult to believe that axonal responses could be distinguished for any length of time by the relatively coarse tips of our pipettes. It should be noted that we do have good evidence of excitation of nerve cell bodies in the cortex by L-glutamate, since all the Betz cells that we have studied (see Krnjević & Phillis, 1962) could be excited in this way, and they were found at the expected depth (no responses to L-glutamate were given by axons of Betz cells in the white matter).

When a micropipette is gradually pushed through the cortex, neuronal activity can often be recorded (cf. Amassian, 1953; Li & Jasper, 1953; Mountcastle, Davies & Berman, 1957; Grüsser, Grützner & Baumgartner, 1958; Towe & Amassian, 1958), especially if the animal is not deeply anaesthetized. If L-glutamate is released continually from one barrel of the micropipette, the number of distinguishable neurones is greatly increased. Thus many cells lying quiescent within recording distance of the tip, most of which are not susceptible to excitation by peripheral stimuli or by the continuous synaptic bombardment suggested by the local slow waves, can nevertheless be brought into activity by L-glutamate. During routine searching for cells, up to about 20 useful units can be found in this way in the course of one track through the cortex. We have not tried to look systematically for the greatest possible number of distinguishable units. This would undoubtedly be much greater; small spikes can always be detected in the background noise, presumably from cells that are either very small or relatively far from the tip (or both).

If we assume that a distinct response can be recorded up to a distance of about 50 μ from the cell (Svaetichin, 1951; Li & Jasper, 1953; Mountcastle *et al.* 1957; Towe & Amassian 1958) the maximal number of neurones within reach of the tip during one track in the post-cruciate gyrus of the cat would be approximately 320 (taking the mean density of neurones as $20/(100\ \mu)^3$; from Ramon-Moliner, 1961). However, L-glutamate probably excites many small cells, whose spikes are likely to be masked by the activity of larger or nearer cells. When the density of units recorded at different depths is compared with the known density of neurons it appears that our method may tend to discriminate against small cells (Krnjević & Phillis, 1963*a*).

Excitation by L-*glutamate*. The characteristic manner in which units are excited is illustrated in Fig. 2. A small amount of L-glutamate causes the cell to give occasional impulses. If one records activity with a long time constant of amplification, it is usually found that these impulses bear a close relation to negative peaks of slow waves of the electrocorticogram, e.g. Fig. 14*H*. With a stronger current of L-glutamate the discharge becomes more rapid, and also more regular (Fig. 2*B*). In the course of one application the frequency nearly always increases with time, probably because the concentration in the tissue does not reach its maximum immediately. Perhaps the most striking feature of glutamate excitation is its short latency of onset (seldom more than 1 sec) and even more rapid termination, typically within 1 sec of the end of the application (e.g. Figs. 2–6, 9, 10, 15, 18, 19). On the other hand, if too much is given the cell fires at an excessive rate which leads quickly to complete block, presumably by depolarization (Fig. 2*C*). Recovery then follows within a few seconds.

As already pointed out, one can easily prove that the excitation is not due to current flow by passing even larger inward currents through other barrels of the same micropipette. The trace in Fig. 2*D* shows that an inward current through another barrel containing AChCl (which would tend to release Cl^- from the pipette) only caused about as much excitation as a current 30 times smaller through the L-glutamate barrel (cf. Fig. 2*A*). Furthermore, L-glutamate is very effective if it is released from the micropipette by pressure injection. In Fig. 3 the upper record was obtained during the iontophoretic application of glutamate from one barrel (by a current of 40 nA), and the lower record when a hydrostatic pressure of 100 mm Hg was applied to another barrel, also containing Na-L-glutamate. Although the second method of release has a somewhat slower action, both in starting and stopping, the effects produced are comparable.

It has been shown that, given a 3 M-AChCl pipette and a tip resistance of 30–20 MΩ, with 100 mm Hg one can expect a flow rate at the tip of 10^{-10} to 10^{-9} ml./sec (Krnjević *et al.* 1963), equivalent to a release of 0·3–3 pmole/sec. With Na-L-glutamate in the barrel conditions are not likely to be grossly different, and the rate of release would therefore be comparable with that caused by an iontophoretic current of 40 nA.

Excitation by related compounds. If any doubts should remain about the potent action of L-glutamate, they can be dispelled by comparing it with that of D-glutamate. Every one of the 200 cortical cells tested proved much more sensitive to the laevo- than to the dextro-isomer. This can be seen in Fig. 4: identical inward currents (60 nA) were passed through barrels containing the L- and D-isomers of glutamate and aspartate, all made up in similar concentrations and at a similar pH (8·5). In spite of

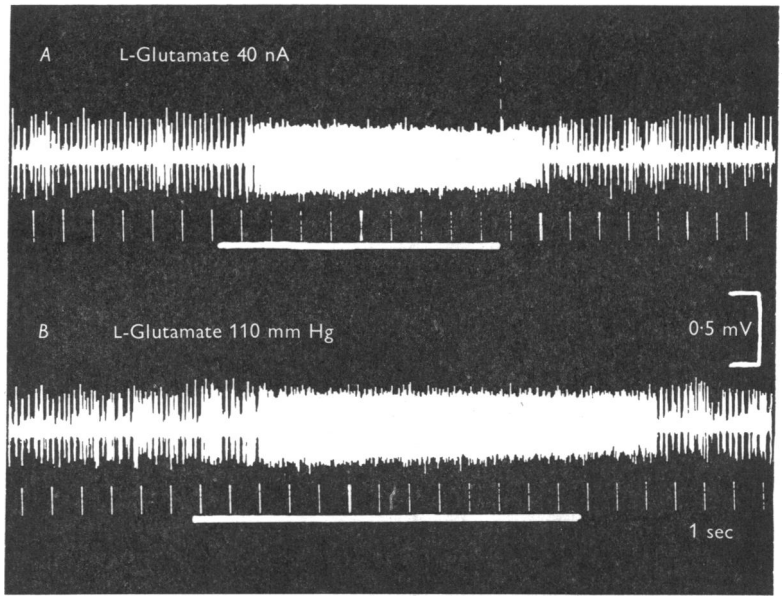

Fig. 3. Excitation by L-glutamate does not depend upon its release by a current. Comparable effects were obtained on unit in cat's cerebral cortex when L-glutamate was released from one barrel of micropipette by iontophoresis (*A*) and from another barrel by applying a head of pressure as indicated (*B*). Both barrels were filled with near-saturated solutions of Na L-glutamate.

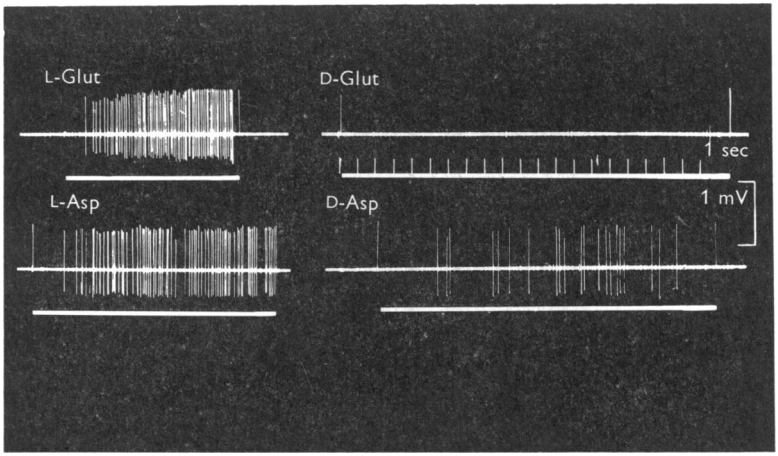

Fig. 4. Comparing actions of L- and D-isomers of glutamate and aspartate on unit in cat's cortex under Dial. Substances were in similar, near-saturated solutions, at pH 8·5; iontophoresis by identical inward currents of 60 nA.

a much longer application, D-glutamate caused only a slight excitation of the cell. Such consistent differences between L- and D-glutamate released by identical currents from solutions at the same pH demonstrate the specific action of the L-isomer, since any artifacts due to current flow, changes in pH etc. would be exactly similar in the two cases. As is shown here, in Fig. 4, D-glutamate was always much less effective than either L- or D-aspartate, but the two forms of aspartate in the aggregate seemed to have approximately the same potency; in some cases the dextro form was more effective, but more often, as in Fig. 4, the laevo form excited a little more strongly. The small variations probably arose from asymmetries in the shape of the micropipette tip.

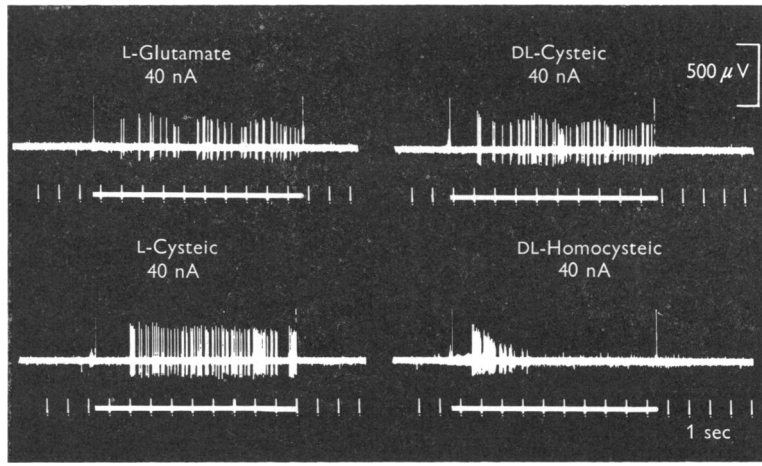

Fig. 5. Sulphonated derivatives of L-glutamate have similar or even stronger excitant actions. All substances were released by iontophoresis near a unit in a cat's cortex under Dial. Note excessive depolarization by homocysteic acid.

However, L-aspartate always had a weaker action than L-glutamate, and the same was true for most other related compounds which might occur in nervous tissues (for instance, glutamine and amino-adipic, amino-pimelic and α-keto-glutaric acids were almost totally inactive): the exception was cysteic acid (either in the DL or L form), whose activity was comparable with or even stronger than that of L-glutamate (Fig. 5). Homocysteic acid (Curtis & Watkins, 1961; Curtis, Phillis & Watkins, 1961*a*) is probably the most strongly exciting substance that we have tested, but it differed from L-glutamate in tending to cause a rapid depolarization block (see Fig. 5) which was not always reversible. Another powerful depolarizing agent is *n*-methyl-D-(or DL) aspartate, known to be particularly effective in causing spreading depression after a topical application (Curtis & Watkins, 1961); this substance was somewhat slower

than L-glutamate in its ability to initiate firing of cortical cells, but the effect usually continued for several seconds after the end of the application (Fig. 6). Moreover, as is also shown in Fig. 6, it tended to cause a prolonged reduction in the sensitivity of the cell to L-glutamate. We have not observed any interference of this kind by other related compounds, such as D-glutamate.

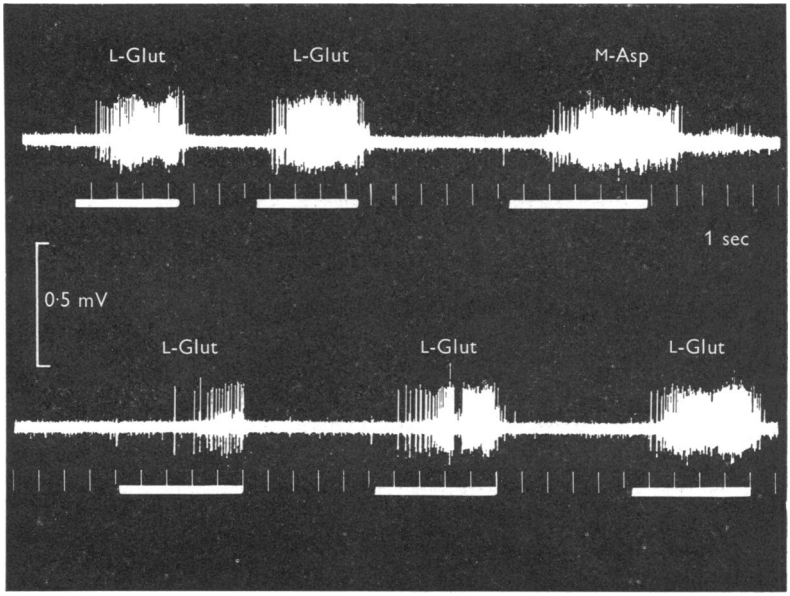

Fig. 6. Unit in cerebral cortex of monkey. Upper line: two applications of L-glutamate (70 nA) followed by one application of *n*-methyl-DL-aspartate (40 nA). Lower line, continuous with above: three more applications of L-glutamate (70 nA) show reduced sensitivity of unit for over 20 sec.

It has been said that dicarboxylic amino acids like L-glutamate have a non-selective *toxic* action on brain cells (Purpura, 1960). Yet one of the most impressive features of this type of excitation is that it can be repeated a great number of times without any evidence of a progressive deterioration in the condition of the cell. Even complete depolarization by an excess of L-glutamate leads only to a transient block (unless the application is continued). If L-glutamate is applied at a very slow rate, just sufficient to cause a cell to fire impulses steadily, the discharge can be maintained for many minutes with no sign of injury or obvious desensitization.

Minimal quantities needed for excitation. Cortical neurones varied greatly in sensitivity (compare Figs. 2 and 4), much of the variation being probably due to differing distances between the tip of the pipette and the

cells; but it appeared that some Betz cells with very large spikes (likely to be large cells) had a relatively high threshold for L-glutamate excitation. This would be in keeping with the observations of Curtis *et al.* (1960), who could not fire spinal motoneurones with L-glutamate.

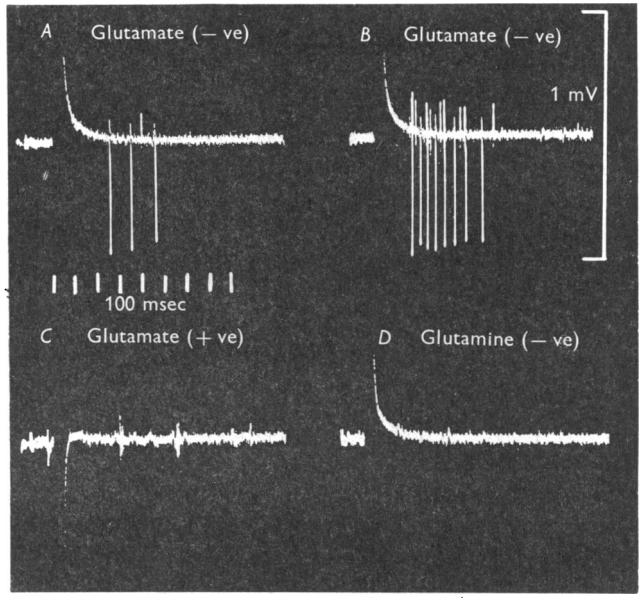

Fig. 7. Two units in cortex of cat under Dial, excited by L-glutamate released by 20 msec pulses of inward current. Voltage applied to pipette was 100 V in *A* and 150 V in *B*. In *C*, polarity of pulse was reversed, and in *D* a similar pulse of inward current was passed through another barrel containing glutamine.

When neurones were very sensitive to L-glutamate, its action could be demonstrated most clearly by inward pulses of current through the pipette. In Fig. 7*A*, a 100 V, 20 msec pulse of L-glutamate (seen as a large artifact near the beginning of the trace) excited two cells, one of them firing three spikes (large) and the other only one spike (small). Both cells fired many more impulses after a 150 V pulse. Outward pulses of 150 V (20 msec) through the same barrel were ineffective, as were inward pulses of the same magnitude through another barrel with a similar resistance but filled with glutamine. Similar results were obtained with several other cells (e.g. Fig. 8*A*, *B*). Under the best conditions, i.e. presumably when the tip was unusually close to the cell membrane, much shorter pulses were effective; the shortest had a duration of only 2 msec. With such pulses, the latency of excitation was reduced to 20–100 msec (cf. Fig. 8*B*). The most sensitive cells could be excited by passing a total charge of about

2×10^{-9} coulomb, corresponding to a release of some 10^{-14} mole of L-glutamate.

The same technique was used to deliver pulses of L- and D-aspartate, with comparable results. The latency of onset was not appreciably longer, and a similar discharge was obtained with somewhat stronger pulses, whereas D-glutamate was again much less effective.

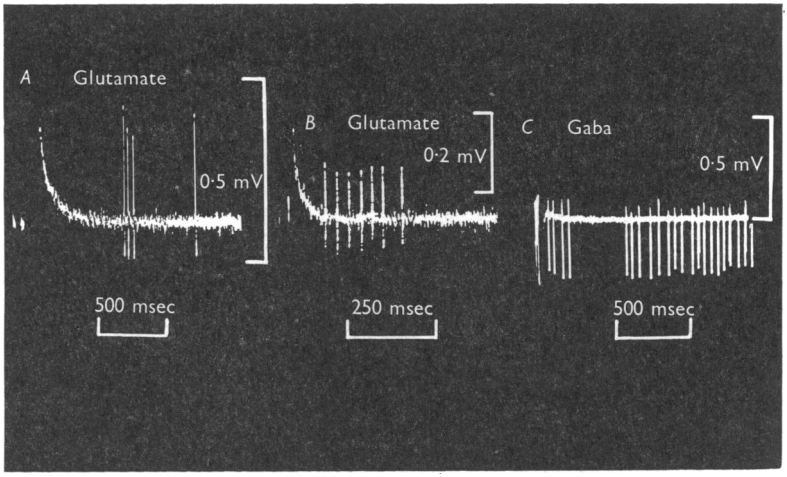

Fig. 8. Two other units in cat cortex under Dial, excited by L-glutamate released by 30 msec (*A*) and 5 msec (*B*) pulses of inward current (about 0·5 μA). Trace in *C* shows transient block of excitation of another unit by GABA released by a 20 msec pulse of outward current. Unit was excited by a background release of L-glutamate from another barrel (60 nA).

Other exciting substances

Amino acids not closely related to L-*glutamate.* The striking inhibitory action on cortical cells of γ-aminobutyric acid (GABA) and several other ω-amino-monocarboxylic acids is described below. Hayashi (1956) suggested that these ω-amino acids have both inhibitory and excitatory properties and that whereas the short-chain compounds (with fewer than 6 carbon atoms) mainly tend to inhibit cortical neurones, those forming a longer chain have a predominantly excitatory action. The later observations by Purpura *et al.* (1959) are consistent with this postulate, but these authors chose to interpret their results in a different way, which led them to believe that all the monocarboxylic ω-amino acids have depressant actions only, the different effects observed being ascribed to a specific blocking of excitatory synapses by the short-chain compounds, and an equally selective blocking of inhibitory synapses by the others.

In our experience the longer-chain ω-amino acids (ϵ-amino-caproic and ω-amino-caprylic, in particular) have proved somewhat ambiguous in

their actions, showing both inhibition and excitation. Of a total of 104 cells tested with these two substances, about one half showed either excitation or depression, the former being somewhat more common with ϵ-amino-caproic, and the latter when ω-amino-caprylic was applied. Examples of both types of actions are given in Fig. 9*A* and *B*. The delayed but maximal firing induced by these compounds (see Fig. 9*B*) is clearly very different from excitation by L-glutamate. It is much more akin to

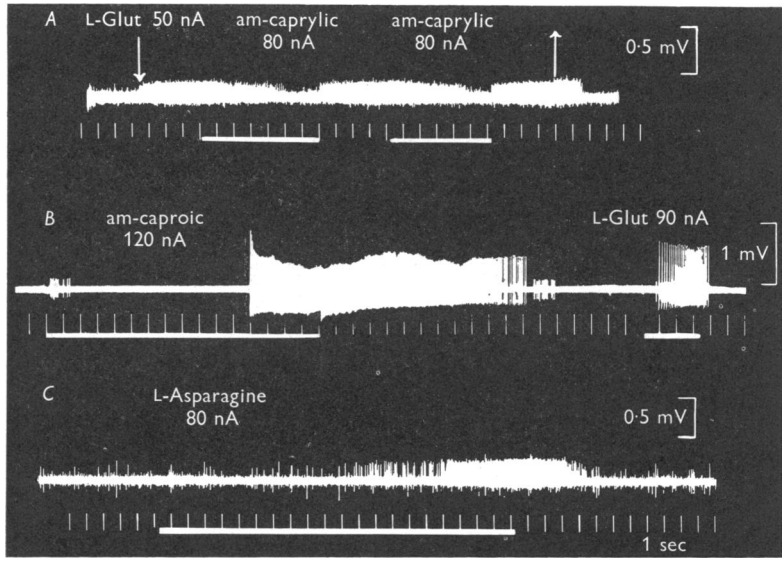

Fig. 9. *A*, *B*: some actions of long-chain ω-amino acids on cortical neurones. *A*: unit in cat cerebellum, at depth of 0·3 mm, during Dial anaesthesia. L-glutamate (50 nA) was applied throughout period between two arrows. During times indicated by white lines, ω-aminocaprylic acid was released as cation (80 nA) causing gradual block of discharge. *B*: 2 units in cerveau isolé of cat, at depth of 0·75 mm. ϵ-aminocaproic acid was released as cation (120 nA) during first signal. Both units were excited, with different latencies, but discharge of small unit was blocked almost immediately. At second signal L-glutamate was released to show that it was still effective. *C*: Excitation of unit in cat's cerebellar cortex under Dial anaesthesia by L-asparagine released as cation (80 nA).

the effects produced by various amines, like 5-hydroxytryptamine (Fig. 10) and nicotine (Fig. 11). The mixed effects observed with the long-chain ω-amino acids are thus fully in agreement with Hayashi's suggestion. On the other hand, we have confirmed the discovery by Purpura *et al.* (1959) and Van Harreveld (1959) of a substantial action of asparagine on cortical cells. Nearly all the 65 neurones tested with L-asparagine (released as a cation from solutions at pH 3·5–4) were excited after relatively long latencies of between 10 and 50 sec (Fig. 9*C*). This effect was observed less regularly with D-asparagine, and only occasionally with glutamine.

Certain amines. Many catechol and indole amines have a depressant action on cortical neurones when released iontophoretically (see below, and Fig. 10*A*). But with relatively large amounts of 5-hydroxytryptamine (5-HT), adrenaline and some other compounds, the cells were sometimes strongly excited instead, as can be seen in Fig. 10 *C* and *D*. This kind of paroxysmal discharge could be produced several times, with no evidence of any permanent damage to the cell. The records in Fig. 10 show that the firing cannot be ascribed to non-specific current actions, or to the creatinine sulphate with which 5-HT is usually associated.

Among other amines of biological interest which were also studied, nicotine requires special mention for its strong, though delayed, excitant

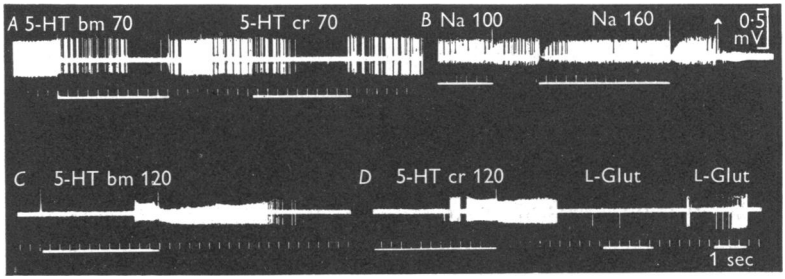

Fig. 10. Some depressant and excitant actions of 5-HT on unit in cerveau isolé of cat, at depth of 0·6 mm in post-cruciate area. *A*: unit excited throughout by a continuous application of L-glutamate (10 nA); discharge was blocked by 5-HT bimaleinate (first signal) and 5-HT creatinine sulphate (second signal), both released from solutions at pH 3·5, by outward currents of 70 nA. *B*: during similar excitation by L-glutamate (continued till arrow), control outward currents of 100 and 160 nA were passed through central, NaCl containing recording channel of multiple pipette (polarizing circuit shunted input thus reducing spikes; polarizing currents also caused temporary block of amplifier). *C* and *D*: Strong excitation of same unit by faster release of 5-HT (120 nA). In *C*, unit was excited 10 sec after starting application of 5-HT bimaleinate; onset of 5-HT creatinine sulphate release was before start of *D*, which shows excitation after 13 sec; two applications of L-glutamate (40 nA) indicate some temporary reduction in sensitivity.

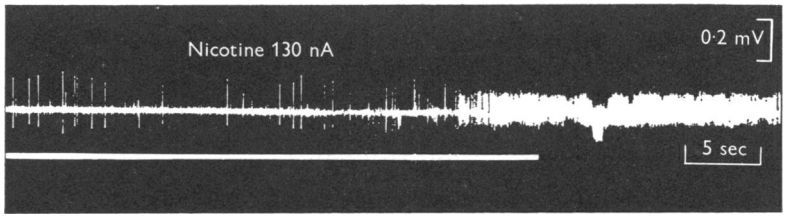

Fig. 11. Unit in cortex of monkey under Dial. Record begun 15 sec after starting iontophoretic application of nicotine as a cation (from a solution of nicotine hydrogen tartrate at pH 4); characteristic paroxysmal discharge went on for another 20 sec. Displacement of base line soon after end of nicotine release was an artifact.

actions observed on 54 cells out of 232 actually tested. Characteristically, after a continuous application of nicotine for 30–60 sec, a neurone would suddenly burst into activity, as in the record illustrated here. Figure 11 shows the last phase of a 45 sec iontophoretic application of nicotine, indicated by the white line below the trace. When firing began it was paroxysmal in a typical manner. The discharge continued for another 20 sec after the end of the trace shown in the figure and, as usual, it was not followed by a considerable period of reduced excitability. This action of nicotine resembles the excitatory action of curare (tubocurarine and dimethyl tubocurarine) also seen on a small number of occasions. Neither of these effects bears any relation to the excitation of certain neurones by ACh; they may help to explain previous observations of excitatory effects of nicotine and curare on the cortex (e.g. Chang, 1953; Rech & Domino, 1960; Morlock & Ward, 1961; Silvette, Hoff, Larson & Haag, 1962).

Acetylcholine. Although large numbers of neurones were tested thoroughly with ACh, as well as other related compounds, only a small proportion could be excited (about 10 % on the average). Most neurones were not affected in any way, showing neither an increase nor a decrease in excitability. The action of ACh was radically different from that of L-glutamate. One need only compare the two traces in Fig. 12 for this to be evident: L-glutamate excitation had the usually sharp onset and abrupt termination; in contrast, excitation by ACh only began after an appreciable interval, during which the spontaneous activity (a feature typical of cholinoceptive cells) tended to be depressed. This initial depression was probably a current effect and could be reproduced with similar outward currents through indifferent barrels. The effect produced by ACh long outlasted its application, as in Fig. 12, the after-discharge going on for 30–60 sec or even more. In this respect cholinoceptive cells in the cerebral cortex were similar to those found in the mid-brain by Curtis & Koizumi (1961).

Like other cortical neurones, cholinoceptive cells were readily excited with L-glutamate. But it was unusual for them to have a very low threshold for the action of L-glutamate, and in several cases the cell was excited much more effectively by ACh than by L-glutamate (cf. Fig. 12).

It is clear that ACh excitation cannot be easily confused with non-specific effects of current flow. ACh^+ was delivered by passing an outward current through the pipette (i.e. with the tip relatively positive). We have shown above that this type of current nearly always tends to depress adjacent neuronal activity (Fig. 1*B*); on the few occasions when an outward current excited a cell, the action was immediate, unlike that of ACh, and it did not persist after stopping the current (Fig. 1*D*).

The distribution and identity of cholinoceptive neurones in the cortex

and their pharmacological properties will be considered in more detail in two subsequent papers. It will be shown that these neurones are related especially closely to ascending pathways arising in the specific rather than the non-specific nuclei of the thalamus, and that the ACh receptors responsible for their activation have strongly muscarinic properties.

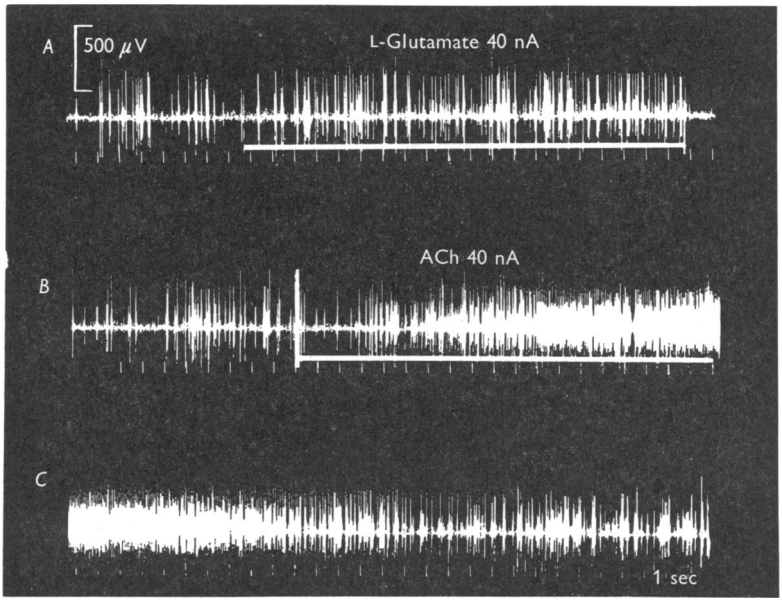

Fig. 12. Comparing excitatory actions of L-glutamate and ACh released iontophoretically by similar inward and outward currents (traces in *B* and *C* are continuous). Units were at depth of 0·95 mm in precruciate area of cat under Dial. Large unit was identified as a Betz cell by antidromic excitation of pyramidal tract.

Hydrogen ions. Most cortical cells can be excited by releasing comparatively large quantities of H^+ in their vicinity. This is probably a non-specific action, similar to the depolarization of peripheral nerves by acid solutions. The action of H^+ is less predictable than that of L-glutamate. The latency of onset is usually much longer and the effect more persistent, as is illustrated in Fig. 13. There is little gradation in the action of H^+ which tends to have an all-or-none character. Cells vary much in their sensitivity to H^+, possibly because of local differences in buffering capacity.

Apart from intrinsic interest, an important reason for the study of H^+ is that it must be released iontophoretically to some extent by outward currents which are passed through acid solutions. It may thus interfere with the action of other substances, or even cause excitation by its own presence. We have examined this latter possibility by studying the

behaviour of nearby cells while passing outward currents from pipettes filled with concentrated NaCl solutions at various levels of pH. Our observations suggest that excitation by H^+ is very unlikely if the internal pH exceeds 2·5, in agreement with previous observations on spinal neurones (Curtis, Phillis & Watkins, 1961*b*).

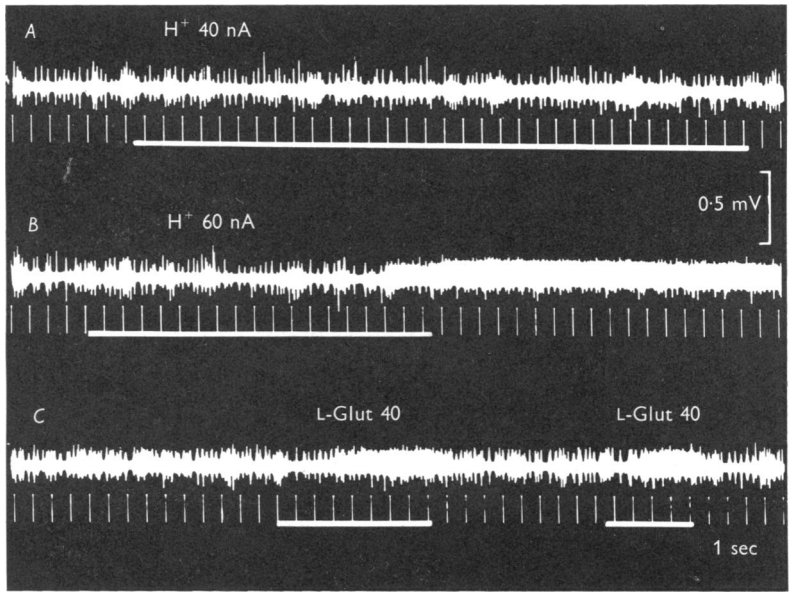

Fig. 13. Effects of H^+ on cerebral cortical unit in cat under Dial. *A*: during signal H^+ was released from 0·1 N-HCl by current of 40 nA. *B*: H^+ now released by current of 60 nA. *C*: record follows 110 sec after end of *B*; it shows remnants of excitation by H^+ and effects of two further applications of L-glutamate.

The excitation produced by nicotine, long-chain ω-amino acids, 5-HT and adrenaline bears some resemblance to that induced by H^+. Since these compounds were all released from acid solutions, we have considered the possibility that they all excite by causing a sufficient increase in tissue acidity. Without excluding this factor entirely, it seems to be relatively unimportant for several reasons. In many cases, as a control, we could release similar substances from equally acid solutions in adjacent barrels: for instance, 5-hydroxytryptophan applied to the same cells, under the same conditions, as 5-HT, did not tend to excite. Effects were obtained even when the solutions were only mildly acid (pH 5). Other amino acids such as leucine, citrulline and tryptophan did not have an excitant action like ϵ-amino caproic acid. Nicotine only excited about a quarter of the cells tested, and usually only after a very long delay; whereas H^+ excites most cells within 10–15 sec. It seems likely that any agent capable of pro-

ducing a sudden break-down in membrane resistance will cause firing of this type.

Cortical neurones are more resistant to OH^- ions, which only sometimes caused a relatively mild depression when applied in very large amounts (iontophoretic currents of > 100 nA for 1 min).

Potassium and some other agents. Potassium may be of significance in the causation of spreading depression (Grafstein, 1956; Marshall, 1959). The extracellular concentration of K in the brain is probably identical with that of the cerebrospinal fluid, i.e. about 2–3 mM (Davson, 1956). If the brain cells behave like peripheral nerves (Stämpfli, 1959), no appreciable change in membrane potential is to be expected unless the external K concentration is at least doubled (cf. Libet & Gerard, 1939) and there may not be much change before it is raised to some 10–20 mM.

A comparison between the excitatory effects of L-glutamate and K^+ released under the same conditions and in the same manner is therefore particularly instructive, since it gives an indication of the probable effective concentration achieved in the vicinity of the cell by iontophoresis, and also gives a clue to the relative contributions of K^+ or L-glutamate in the causation of spreading depression if both agents are released by depolarized cells.

This experiment was done on sixteen cells. A typical example is given in Fig. 14, which shows a unit found in the posterior sigmoid gyrus, firing spontaneously in close relation to local slow waves (*H*) and also in response to a peripheral stimulus (*G*). It was readily excited within a few seconds by L-glutamate (*A*, *B*) and ACh (*C*, *D*) but K^+ applied with a much larger current failed to cause more than a relatively slow and irregular discharge, even after a period of 90 sec. The majority of cells showed no appreciable excitation by K released in similar quantities, and in no case did K induce a cell to fire rapidly.

Unlike K^+, NH_4^+ consistently had no action at all on the neurones tested, in agreement with the observations of Hillman & McIlwain (1961).

On the other hand, many neurones were excited quite strongly by EDTA (diaminoethane tetra-acetic-acid) (Fig. 15), normally after a relatively short latency when compared with its action on spinal neurones (Curtis, Perrin & Watkins, 1960). However, EDTA, which was released as an anion, was always less effective, and often very much less so than L-glutamate (cf. Fig. 15*C*, *D*).

Inhibitory substances

γ-Aminobutyric acid (GABA). The inhibitory action of GABA on cortical neurones is just as remarkable as the excitatory action of L-glutamate. The two substances complement each other very satisfactorily; both are universally effective, having quick actions that are rapidly reversed, with no

apparent damage to the cells, so that they can be safely applied many times. The amounts of GABA required to cause marked inhibition have varied between 4 and 100 nA of outward current, values of 20–50 nA being most common. When cells were particularly sensitive, it was possible to demonstrate its quick action by passing pulses of outward current through the pipette. For instance, in Fig. 6*C* a 20 msec pulse of GABA at the start

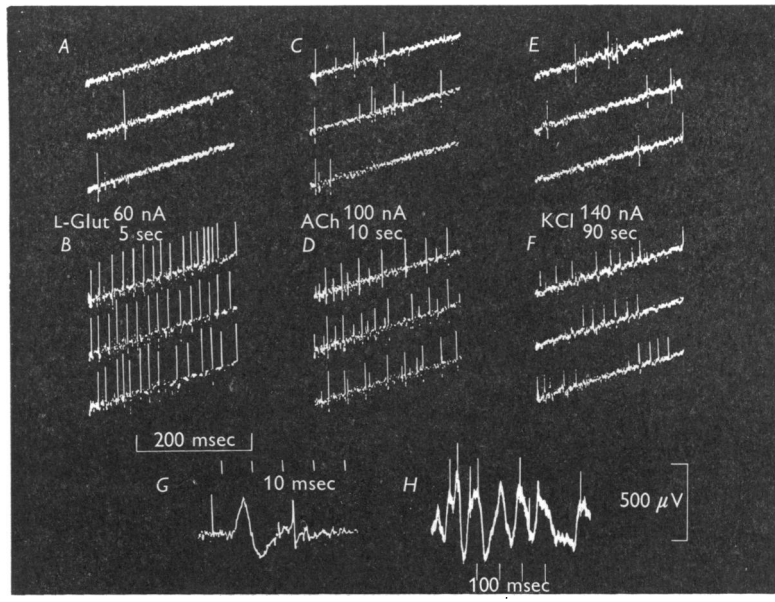

Fig. 14. Comparing effects produced by iontophoretic release of L-glutamate, ACh and K ions. Unit was found at depth of 1·1 mm in post-cruciate area of cat anaesthetized with Dial: it discharged impulses spontaneously in close relation to negative peaks of slow waves (*H*) and could be activated by electrical stimulation of contralateral forepaw (*G*; recorded with short time constant of amplification). *A*, *C* and *E* are control records of oscilloscope sweeps at 4/sec. *B*, *D* and *F* were taken at various times after starting release, as indicated. Some spikes have been retouched.

of the trace caused a transient block of the continuous discharge of a cell induced by the steady release of L-glutamate from another barrel of the same pipette. The time course of the inhibition is not unlike that of the excitation caused by comparable pulses of L-glutamate, as would be expected if both effects were simply dependent on the concentration in the tissue.

As in some other tissues (Curtis *et al.* 1959; Edwards & Kuffler, 1959), the effect of GABA is initially at a maximum, and then diminishes appreciably after several seconds during a prolonged application, owing to desensitization or an increased rate of inactivation.

The interaction between L-glutamate and GABA is shown even more clearly in Fig. 16, where 9 pulses of GABA (visible in the record as prominent vertical artifacts) were responsible for regular interruptions of the rapid firing produced by a continuous release of L-glutamate (indicated by the white line below the trace).

GABA was usually released as a cation by passing an outward current through a concentrated solution at pH 3·5–4 (pK_1 = 4·23). It has been

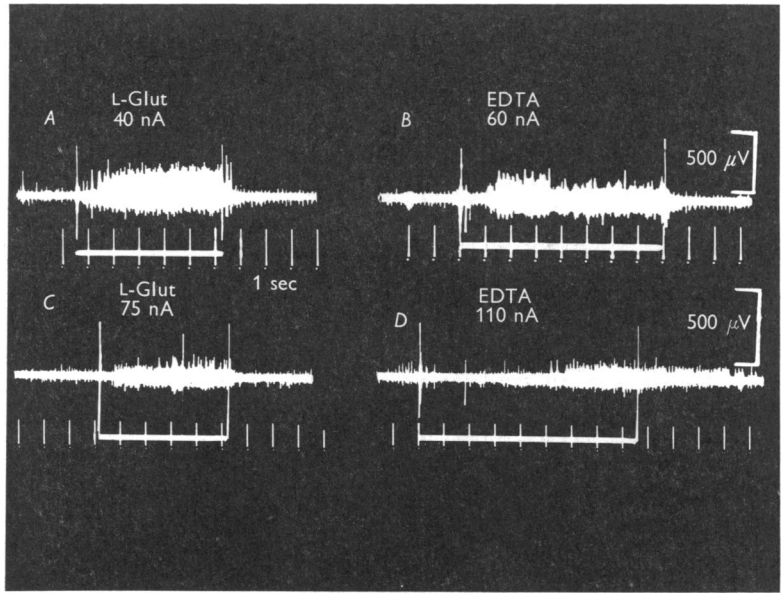

Fig. 15. Excitation by EDTA, released as anion from near saturated solution of di-sodium salt. Both units were in post-cruciate cortex of a cat, anaesthetized with Dial, and they were excited even more effectively by L-glutamate; compare *A* with *B*, and *C* with *D*.

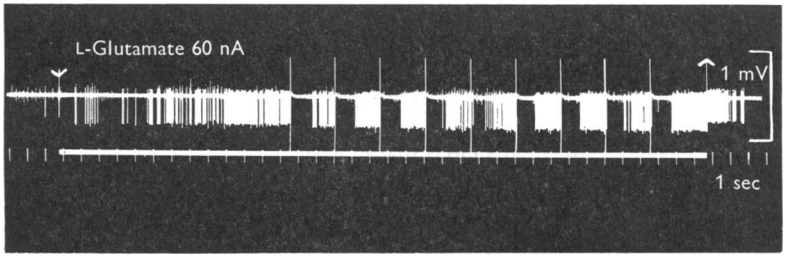

Fig. 16. Iontophoretic release of L-glutamate (between two arrows) initiated a continuous discharge of unit in cortex of cat under Dial; excitation was blocked nine times by GABA released from another barrel by 20 msec pulses of outward current (indicated by large electrical artifacts).

suggested that in its inhibitory properties, GABA released iontophoretically as a cation may differ substantially from the zwitterion, the predominant form at a body pH (Kuno, 1961; Muneoka, 1961). This criticism is probably unjustified, because inhibition is obtained easily if GABA is released either by pressure injection or by an outward current, from a pipette containing a neutral solution. The latter may seem surprising since GABA is at its iso-electric point at pH 7·5. The probable explanation is that in the iso-electric region GABA solutions in contact with hard glass have a substantial zeta potential, reaching a peak of 36–38 mV between pH 7 and 10 (unpublished observations). This gives an electro-osmotic mobility of 3 μcm/V sec, which would easily account for a significant release of neutral GABA (cf. Krnjević *et al.* 1963). It is not possible to give at present a strictly quantitative comparison of GABA cations and zwitterions, but our results do not indicate any marked difference between their

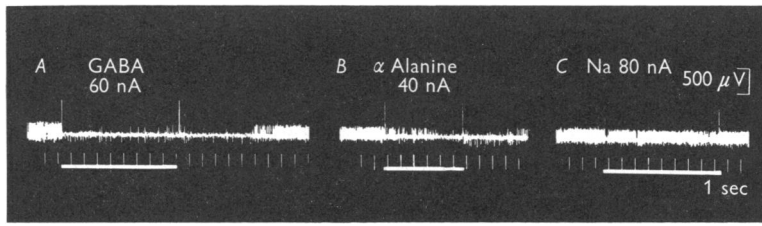

Fig. 17. Block of fast spontaneous discharge of cerebellar unit, in cat under Dial, by iontophoretic release of GABA and α-alanine from solutions at pH 3·5. An even larger outward current through another barrel containing Na salt of cysteic acid had only a slight depressant effect (*C*).

actions. The region of high zeta potentials is distributed very asymmetrically about the iso-electric point of GABA, being much more pronounced on the alkaline side of pH 7; this probably accounts for the difficulty experienced in trying to release GABA as an anion from alkaline solutions (Curtis & Watkins, 1960).

GABA is just as effective in blocking the natural activity of a neurone, i.e. activity arising 'spontaneously' or from the excitation of various pathways, as that initiated by L-glutamate. The fast spontaneous discharge of the unit illustrated in Fig. 17 was temporarily stopped by the iontophoretic release of GABA (*A*); as a control, a somewhat larger current was passed through another barrel of the same pipette, with no significant effect (*C*).

We have tested GABA on some 400 neurones including several Betz cells, in most areas and at various depths of the cerebral and cerebellar cortex, with and without anaesthesia; apart from one or two doubtful responses, we have never failed to observe a clear inhibition. Betz cells

were identified by short latency antidromic activation from the bulbar pyramids (see Krnjević & Phillis, 1963*a*). Such a widespread action is not consistent with the claim that GABA only blocks superficial, excitatory axodendritic synapses (Purpura, 1960). Although in our experiments GABA prevented the excitation of Betz cells by L-glutamate and by synaptic activity, antidromic invasion was not readily blocked.

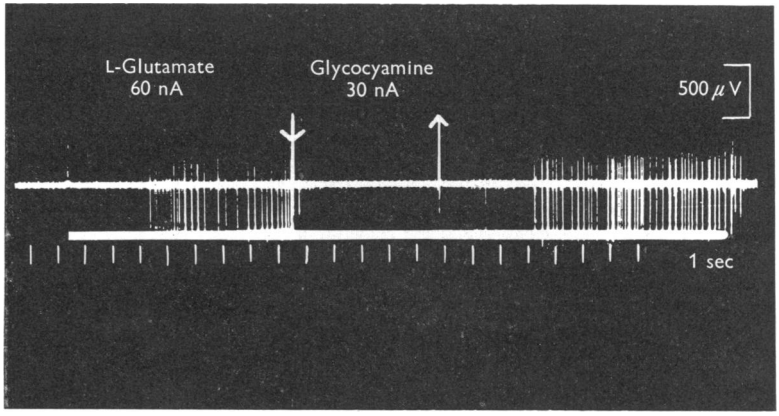

Fig. 18. Depressant action of glycocyamine (guanidino-acetic acid) on excitation by L-glutamate; both substances were released iontophoretically, by outward and inward currents, respectively. Unit in cortex of cat under Dial.

Other inhibitory substances

Amino acids related to GABA. Several close derivatives, including γ-aminocrotonic, β-hydroxy-γ-aminobutyric, δ-aminovaleric acids and β-alanine (but not taurine), as well as the ω-guanidine compounds, guanidino-acetic (Fig. 18) and β-guanidino-propionic (but not γ-guanidino-butyric) acids all have inhibitory activities comparable with that of GABA. The strong action of some guanidino-amino acids confirms previous observations on the cat's cortex (Purpura *et al.* 1959), the toad's spinal cord (Curtis *et al.* 1961*a*) and the crayfish stretch receptor (Edwards & Kuffler, 1959), but it is in marked contrast with their reported inactivity on cat spinal neurones (Curtis & Watkins, 1960). Even amino acids which are less closely related, such as α-alanine (Fig. 17*B*) and glycine are appreciably active. But the long chain ω-amino acids have only a relatively weak and inconstant inhibitory action, often masked by excitatory effects (Fig. 9). We have also looked for a specific block of inhibitory synapses by these substances (cf. Purpura *et al.* 1959). Surface stimulation with single shocks (10 V) causes a strong inhibition of many cells in the cortex, lasting over 100 msec. This could not be reduced by large amounts of ϵ-aminocaproic acid given by iontophoresis or by lavish surface appli-

cation of a 1 % solution. It is interesting that with regard to the actions of inhibitory amino acids, cortical neurones in general behave remarkably like crayfish stretch receptors (Edwards & Kuffler, 1959).

Some indole and catechol amines. Many compounds in this group have been applied to cortical neurones iontophoretically (mostly as cations, released from acid solutions). A systematic survey cannot be given here, but it should be noted that, in agreement with previous observations by Marrazzi (1961), there was a general tendency towards a depression of the activity of most cells (although large doses sometimes caused excitation,

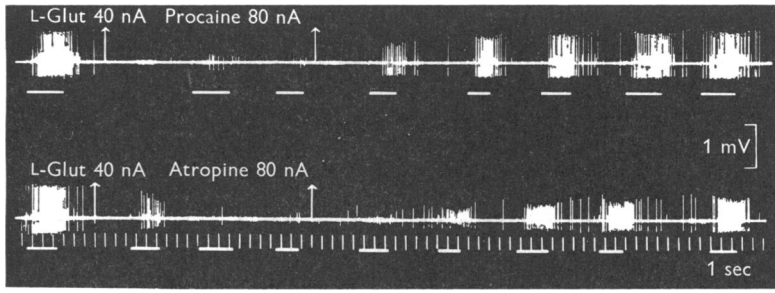

Fig. 19. Comparing depressant actions of similar iontophoretic currents of procaine and atropine on same cortical unit in cat under Dial; periods of release are indicated by two arrows. Excitability was tested repeatedly by applying L-glutamate during times shown by horizontal white lines below traces.

as described above). The depressant action was usually shown by first exciting units with L-glutamate. Among the most effective compounds were ephedrine, dopamine and 5-HT (see Fig. 10), while somewhat weaker depressions were seen with adrenaline, noradrenaline, amphetamine and histamine. Even the most potent were much less active than GABA.

In contrast to these comparatively short-lasting effects, LSD (lysergic acid diethylamide) and several derivatives (cf. Curtis & Davis, 1962) had a depressant action that was only slowly reversible (full recovery required a period of at least 30 sec); this effect was rather similar to the general blocking action of atropine described below.

Procaine, atropine and hyoscine. Like spinal neurones (Curtis & Phillis, 1960), most cortical units are readily depressed by atropine and hyoscine released iontophoretically. An example is given in Fig. 19, which shows clearly the associated characteristic reduction in spike size, an effect that persists for 30–60 sec after the end of the application. This blocking action of atropine is no doubt due to its local anaesthetic properties, and it is, indeed, almost identical in its time course with the effects produced by a similar iontophoretic current of procaine (Fig. 19). But procaine, unlike atropine, does not cause a prolonged interference with the size of the spike.

This type of depression by atropine and hyoscine is fully reversible within 1 min and it bears no relation to the ACh sensitivity of certain cells. It must therefore be sharply distinguished from the specific, prolonged block by atropine of the excitation of cholinoceptive neurones by ACh (Krnjević & Phillis, 1962, 1963*b*).

DISCUSSION

Current effects

Our observation that outward currents (with micropipette tip relatively positive) in general tend to depress neuronal activity, while inward currents have the opposite action, are in agreement with some previous reports based upon studies with micropipettes of comparable tip dimensions (Burns & Salmoiraghi, 1960; Curtis & Koizumi, 1961). The effect is not surprising, since the inward current flow will tend to depolarize the nearer portions of the neurone, and this may be enough to trigger spikes.

With very fine micropipette tips it is possible to approach much closer to the cell. If the tip is moved until the recorded spike becomes nearly positive, it is likely that the tip is now pushing against the cell membrane and causing local inactivation. Under these conditions the changes observed should resemble those seen when current flows into a cell from an intracellular electrode, i.e. an outward current depolarizes and therefore excites the neurone and vice versa (Strumwasser & Rosenthal, 1960). This effect will be only seen occasionally with large tips, which are very unlikely to approach a cell sufficiently without causing fatal damage, as in the present study. It is therefore very surprising that Spehlmann & Kapp (1961) have found consistently this kind of behaviour while studying neurones in the visual cortex of the cat with pipettes comparable to ours.

Tasaki, Polley & Orrego (1954) have calculated that the extracellular spikes recorded from cells in the visual cortex are consistent with an external current flow in the order of 10^{-7} A (for a 10 μ cell). This is similar to the currents which often caused appreciable changes in the cellular firing when applied from micropipettes and would seem to support the belief that the excitability of cells can be altered by adjacent neuronal activity (Bremer, 1941; Gerard, 1942).

Actions of amino acids

Possible transmitter functions. One cannot fail to be impressed by the powerful complementary actions of L-glutamate and GABA. These substances, together with glutamine, form a very closely related trio, potentially capable of a full control of neuronal activity. Glutamine itself is rather inactive and so suitable for storage; L-glutamate apparently excites all cortical neurones in a manner which lends itself to fine gradations of

intensity and duration, without desensitization or damage when applied repeatedly or for a prolonged period; while GABA inhibits neurones no less effectively and with a similar flexibility. It is therefore all the more significant that all three compounds are not only present in the brain in abundance but are very common currency in the cerebral metabolic exchanges (McKhann, Albers, Sokoloff, Mickelson & Tower, 1960; Albers, 1960).

Clearly, in addition to the substantial quantities of L-glutamate and GABA normally present in the cortex, a copious supply is thus assured from metabolic processes. Although there is no conclusive evidence at present that glutamate and GABA function in the brain as excitatory and inhibitory transmitters, it is obvious that their quick and reversible actions and ready availability make them eminently suitable for such roles.

Glutamate in the cortex. The normal L-glutamate content of the mammalian brain is about 10 μ-mole/g (Berl & Waelsch, 1958; Tower, 1960). If this was evenly distributed in the cerebral tissues, the concentration in the extracellular fluid would be approximately 10 mM. This is certainly much more than the threshold concentration for excitation and would probably keep the cerebral neurones permanently depolarized (cf. Hillman & McIlwain, 1961).

The iontophoretic method does not permit a very accurate estimate of concentration in the tissues, but it is possible to calculate approximately the probable concentration obtained by diffusion at various distances from the tip of the pipette, taking the latter as an instantaneous or a continuous point source, in an infinite homogeneous medium (del Castillo & Katz, 1955; Carslaw & Jaeger, 1959; Curtis *et al.* 1960).

The analysis based on the continuous source (with constant current) is of limited value, because we have little indication of the distance between the pipette tip and the neurone under observation. When short pulses of current are used, the tip of the pipette becomes an 'instantaneous' point source, and there is a simple relation between the time at which the maximum concentration is reached at any point, and its distance from the source (as pointed out by del Castillo & Katz, 1955). Thus, in view of the extremely rapid action of L-glutamate it is a reasonable assumption that the peak concentration coincides with the peak of the neuronal response; we can then calculate both the concentration and the distance between the tip and the neurone. The peak maximum concentration is given by $C = \frac{Q \exp(-1{\cdot}5)}{8\,(\pi DT)^{1{\cdot}5}}$, where Q is the amount of substance released 'instantaneously', D its diffusion coefficient and T the time to the peak.

If we take as an example the response to a pulse of L-glutamate shown in Fig. 7*A*, the total charge passed through the pipette was about 10^{-8} C. Taking the transport number for glutamate as 0·5, the value of Q is about 5×10^{-14} mole. The coefficient of diffusion of glutamine in aqueous solutions at 25° C is $7{\cdot}62 \times 10^{-6}$ cm²/sec (Longsworth, 1953). If we assume that glutamate diffuses at a similar rate, and make allowances for the higher temperature (a factor of about 1·5) and for the slowing of diffusion by various obstructions in the brain (a factor of about 2, cf. McLennan, 1957), the probable effective diffusion coefficient is approximately 5×10^{-6} cm²/sec. From Fig. 7*A* it is clear that the peak response occurs at

about 0·3 sec: calculating C in the above equation thus gives a value of $1{\cdot}4 \pm 10^{-7}$ mole/ml. or 0·14 mM. The corresponding diffusion distance is obtained from $l = \sqrt{6DT}$, i.e. 30 μ in this instance. Similar calculations for the responses shown in Fig. 8*A* and *B* gave values of 0·095 mM for both peak concentrations and 47 and 25 μ for the respective distances, while the comparable discharge of another cortical unit, in a monkey, corresponded to a peak concentration of 0·12 mM and a diffusion distance of 17 μ. It is clear that the calculated effective concentrations fall within a fairly narrow range. Moreover, the calculated diffusion distances are well inside the limits beyond which substantial spikes are not likely to be recorded.

Glutamate is not likely to diffuse freely in all directions in the brain, as in the theoretical model. Although there is almost no true extracellular space in the brain, a substantial fraction (0·2–0·5) of the total volume, possibly made up of astroglia, is relatively accessible to several 'extracellular' ions (De Robertis & Gerschenfeld, 1961). If we assume that glutamate also moves freely through this space, and make the appropriate correction, the true tissue concentrations of L-glutamate which caused a fast discharge in our experiments were probably in the region of 0·5–1·2 mM. The weak and inconstant excitation produced by prolonged applications of K currents tenfold greater give some support to this analysis, since an increment of 5–10 mM in external K is likely to be the minimum required for an appreciable depolarization.

L-glutamate can therefore only be stored in the brain inside cells. If we take the intracellular fluid fraction as 0·5–0·8 the amount of L-glutamate, inside an average nerve cell (with a volume of 6620 μ^3 according to Ramon-Moliner, 1961) would be $13{\cdot}2\text{–}8{\cdot}3 \times 10^{-14}$ mole. The smallest amount needed to excite a cell by iontophoresis was about 10^{-14} mole.

Glutamate in spreading depression. Our observations evidently support the suggestion that spreading depression may be caused by the liberation of intracellular glutamate (Van Harreveld, 1959), although K might also leak out of depolarized cells, and so contribute to the general spread of depolarization. According to Curtis & Watkins (1961) spreading depression is elicited most effectively by *n*-methylaspartate. This can be accounted for by its characteristic prolonged action (though relatively slow in onset) and the fact that it is probably not metabolized rapidly. The depression of the sensitivity of the neurones to L-glutamate, which persists for as much as 30 sec after a short iontophoretic application of *n*-methyl-DL-aspartate (cf. Fig. 6), may be an important additional factor if L-glutamate is concerned in normal transmission. These features strongly suggest that the binding between *n*-methyl-DL-aspartate and the glutamate receptor is only slowly reversible; its action would therefore in some respects be analogous to that of decamethonium at peripheral cholinergic synapses (del Castillo & Katz, 1957).

Mechanism of L-glutamate and GABA actions. Although it has been

suggested that L-glutamate and GABA act mainly inside cells (Elliott, 1960; Hillman & McIlwain, 1961) their actions are so rapid (with latencies corresponding to the expected diffusion times) as to be necessarily in the region of the surface membrane. In any case, since there is already so much glutamate and GABA inside, the entry of a fraction of 10^{-14} mole would not change the intracellular content significantly. It is known that cells in general can accumulate amino acids against a concentration gradient (Christensen, 1960), and the uptake of L-glutamate by cortical cells has been demonstrated under various conditions (Stern, Eggleston, Hems & Krebs, 1949; Tower, 1960). The simplest explanation for the action of L-glutamate is one which would account for both its transport into the cell, and the simultaneous apparent increase of Na^+ permeability. If we postulate the existence in the membrane of a carrier molecule with a resultant positive charge, capable of reacting with glutamate, it is not too difficult to suppose that this positive charge may be so placed as to prevent the inward movement of Na^+. Combination with L-glutamate might then allow an appreciable influx of Na^+ until the return of the carrier to its original position. This mechanism would ensure the rapid removal of glutamate from the external fluid and thus help to explain the lack of an after-discharge. The inward movement of the carrier is itself apparently related to the Na^+ influx in some way, since glutamate accumulation ceases in the absence of external Na^+ (Takagaki, Hirano & Nagata, 1959).

We know little about the properties of the L-glutamate receptor (? carrier). Our results are consistent with the hypothesis of a 3-point receptor for excitatory amino acids, with fairly rigid steric specificity (Curtis & Watkins, 1960). In fact, cortical neurones seem to be much more discriminating in this respect than spinal neurones, which are almost equally sensitive to L- and D-glutamate, according to the same authors. Although D-glutamate is much less active, this is apparently compensated by the slow rate of its utilization by the tissue (Weil-Malherbe, 1936; Stern *et al.* 1949; Takagaki *et al.* 1959) so that a little may go a great deal further, especially in the initiation of spreading depression (Van Harreveld, 1959). Unlike *n*-methyl-DL-aspartate, it does not interfere markedly with the action of L-glutamate, presumably because of its low affinity for the receptor. If the postulated carrier molecule is, or includes, a metal ion, the substantial excitant action of EDTA would be readily understood.

In the case of the neutral, short-chain ω-amino acids, we are dealing presumably with a different carrier, which may be involved in the accumulation of GABA by cortical slices, demonstrated by Elliott & van Gelder (1958). Amino-acid transport is said to be closely related to the accumulation of K (Christensen, 1960) so that it is conceivable that movements of the ω-amino-acid carrier are somehow associated with the opening of

smaller channels, permeable to K^+ and possibly Cl^- but not Na^+, which would tend to stabilize the membrane potential near its resting level and so reduce excitability.

Actions of other substances. The most common action of the various indole and catecholamines has been a depression of neuronal activity, in agreement with previous observation on the effects of intracarotid injections (Marrazzi, 1961); but all these compounds were less effective than GABA (even dopamine, cf. McGeer, McGeer & McLennan, 1961). It seems a remarkable fact that neurones in some other parts of the central nervous system are apparently quite insensitive to all these drugs (e.g. neurones in the mid-brain and the medulla, Curtis & Koizumi, 1961, and in the spinal cord, Curtis, Phillis & Watkins, 1961*b*; Curtis, 1962); even in the lateral geniculate body several of these compounds block synaptic transmission but not excitation by L-glutamate (Curtis & Davis, 1962). The ease with which the firing of cortical neurones by L-glutamate is depressed by 5-HT and other compounds suggests that these substances do not cause a specific block of all synaptic transmission in the brain.

SUMMARY

1. Many substances have been applied to single units in the cerebral and cerebellar cortex of cats, rabbits and monkeys, under various conditions of anaesthesia, by iontophoretic release and, in some cases, also by pressure injection from multibarrelled micropipettes.

2. L-glutamate and several related amino acids (but not D-glutamate) excited all neurones quickly and powerfully. The effect was almost instantly reversible and could be obtained with minute amounts of excitant (10^{-14} mole). Repeated or prolonged applications did not lead to desensitization.

3. Other amino acids with weaker excitatory actions included long-chain ω-amino monocarboxylic acids and asparagine; glutamine was very ineffective.

4. A small proportion of neurones were excited in a characteristic manner by ACh: the onset of firing was slow, and there was a prolonged after-discharge.

5. General excitation was also caused sometimes by curare and nicotine (this effect was not related to ACh-sensitivity), by H^+, EDTA, and relatively large doses of compounds like 5-HT and adrenaline.

6. GABA and several other short-chain ω-amino acids, as well as some ω-guanidino derivatives, had a powerful and quick depressing action on all neurones, comparable in effectiveness with the exciting action of L-glutamate.

7. The excitability of all cortical neurones could be blocked temporarily with procaine, atropine and hyoscine, recovery taking place within 30–60 sec.

8. Various indole and catechol amines, such as ephedrine, dopamine, LSD, 5-HT adrenaline and amphetamine also depressed neuronal activity in a reversible manner.

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